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| (21) International Application Number: PCT/US86/02227 (22) International Filing Date: 21 October 1986 (21.10.86) (31) Priority Application Number: 798,459 (32) Priority Date: 15 November 1985 (15.11.85) (33) Priority Country: US (60) Parent Application or Grant (63) Related by Continuation US 798,459 (CIP) Filed on 15 November 1985 (15.11.85) (71) Applicant (for all designated States except US): THE UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US). | | (72) Inventor; and (75) Inventor/Applicant (for US only) : TEN BRINK, Ruth, E. [US/US]; 1713 Golfview, Kalamazoo, MI 49001 (US). (74) Agent: WELCH, Lawrence, T.; Patent Law Depart- ment, The Upjohn Company, Kalamazoo, MI 49001 (US). (81) Designated States: AT (European patent), BE (Euro- pean patent), CH (European patent), DE (European patent), FR (European patent), GB (European pa- tent), IT (European patent), JP, LU (European pa- tent), NL (European patent), SE (European patent), US. Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> |
| (54) Title: NOVEL RENIN INHIBITING POLYPEPTIDE ANALOGS CONTAINING S-ARYL-D- OR L- OR DL- CYSTEINYL, 3-(ARYLTHIO)LACTIC ACID OR 3-(ARYLTHIO)ALKYL MOIETIES (57) Abstract Novel renin-inhibiting peptides of the formula X-A ₆ -B ₅ -C ₈ -D ₉ -E ₁₀ -F ₁₁ -G ₁₂ -H ₁₃ -I ₁₄ -Z, wherein X and Z are terminal groups, C ₈ contains S-aryl-D- or L- or DL-cysteinyl, 3-(arylthio)lactic acid, and 3-(arylthio)alkyl moieties, and the remain- ing variables are amino acid residues. Such inhibitors are useful for the diagnosis and control of renin-dependent hyper- tension. | | |

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NOVEL RENIN INHIBITING POLYPEPTIDE ANALOGS CONTAINING
S-ARYL-D- OR L- OR DL-CYSTEINYL, 3-(ARYLTHIO)LACTIC ACID
OR 3-(ARYLTHIO)ALKYL MOIETIES

DESCRIPTION

5 BACKGROUND OF THE INVENTION

The present invention provides novel compounds. More particularly, the present invention provides novel renin-inhibiting peptide analogs. Most particularly, the present invention provides renin-inhibitory compounds having S-aryl-D- or L- or DL-cysteinyl, 3-(arylothio)lactic acid or 3-(arylothio)alkyl moieties at the 8 position (as compared to the renin substrate described below). The renin inhibitors provided herein are useful for the diagnosis and control of renin-dependent hypertension.

Renin is an endopeptidase which specifically cleaves a particular peptide bond of its substrate (angiotensinogen), of which the N-terminal sequence in equine substrate is for example:

| | | | | | | | | | | | | | | | | | |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|-----|-----|-----|-----|--|--|----|
| | | | | | | | | | | Renin | | | | | | | |
| | | | | | | | | | | ↓ | | | | | | | |
| | Asp | Arg | Val | Tyr | Ile | His | Pro | Phe | His | Leu | Leu | Val | Tyr | Ser | | | IA |
| 20 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | | | |

as found by L.T. Skeggs et al, J. Exper. Med. 106, 439 (1957). Human renin substrate has a different sequence as recently discovered by D.A. Tewkesbury et al, Biochem. Biophys. Res. Comm. 99, 1311 (1981). It may be represented as follows:

Renin
↓
-Val-Ile-His-
11 12 13 IB

30 and having the sequence to the left of the arrow (\rightarrow) being as designated in formula 1A above.

Renin cleaves angiotensinogen to produce angiotensin I, which is converted to the potent pressor angiotensin II. A number of angiotensin I converting enzyme inhibitors are known to be useful in the treatment of hypertension. Inhibitors of renin are also useful in the treatment of hypertension.

INFORMATION DISCLOSURE

A number of renin-inhibitory peptides have been disclosed. Thus, U.S. patent 4,424,207, and European published applications 45,665 and 104,041 disclose certain peptides with the dipeptide at the 10,11-position containing an isostere bond. A number of statine derivatives stated to be renin inhibitors have been disclosed, see, e.g., European published applications 77,028; 81,783; and 114,993; and U.S. patents 4,478,826; 4,470,971 and 4,479,941. Terminal disulfide cycles have also been disclosed in renin inhibiting peptides; see, e.g., U.S. patents 4,477,440 and 4,477,441. Aromatic and aliphatic amino acid residues at the 10,11 position of the renin substrate are disclosed in U.S. patent 4,478,827. C-terminal amide cycles are disclosed in U.S. patent 4,485,099. Certain tetrapeptides are disclosed in European publications 111,266 and 77,027. Further, European published application No. 118,223 discloses certain renin inhibiting peptide analogs where the 10-11 peptide link is replaced by a one to four atom carbon or carbon-nitrogen link. Additionally, Holladay et al., in "Synthesis of Hydroxyethylene and Ketomethylene Dipeptide Isosteres", Tetrahedron Letters, Vol. 24, No. 41, pp. 4401-4404, 1983 disclose various intermediates in a process to prepare stereo-directed "ketomethylene" and "hydroxyethylene" dipeptide isosteric functional groups disclosed in the above noted U.S. Patent No. 4,424,207.

Additionally, published European Applications 45,161 and 53,017 disclose amide derivatives useful as inhibitors of angiotensin converting enzymes.

SUMMARY OF THE INVENTION

The present invention particularly provides a renin inhibitory peptide of the formula $X-A_6-B_7-C_8-D_9-E_{10}-F_{11}-G_{12}-H_{13}-I_{14}-Z$,

wherein X is

- (a) hydrogen,
- (b) C_1-C_3 alkyl
- (c) $R_5-O-CH_2-C(O)-$,
- (d) $R_5-CH_2-O-C(O)-$,
- (e) $R_5-O-C(O)-$,
- (f) $R_5-(CH_2)_n-C(O)-$,
- (g) $R_4N(R_4)-(CH_2)_n-C(O)-$,
- (h) $R_5-SO_2-(CH_2)_q-C(O)-$,

-3-

(i) $R_5-SO_2-(CH_2)_q-O-C(=O)-$, r (j) $R_6-(CH_2)_i-C(=O)-$;

wherein A_6 is absent or a divalent moiety of the formula XL_1 , XL_2 , or XL_{2a}

5 wherein B_7 is absent or a divalent moiety of the formula XL_b

wherein C_8 is absent or a divalent moiety of the formula XL_1 or XL_2 ,

wherein D_9 is absent or a divalent moiety of the formula XL_3 or XL_{2a} ,

10 wherein $E_{10}-F_{11}$ is a divalent moiety of the formula XL_5 , XL_{5a} , XL_{5b} , XL_{5c} , XL_{5d} , or XL_{5e} ,

wherein * indicates an asymmetric center which is either in the R or S configuration;

wherein G_{12} is absent or a divalent moiety of the formula XL_4 or

15 XL_{4a}

wherein H_{13} is absent or a divalent moiety of the formula XL_4

wherein I_{14} is absent or a divalent moiety of the formula XL_5

wherein Z is

(a) $-O-R_{10}$,

20 (b) $-N(R_4)R_{14}$, or

(c) C_4-C_8 cyclic amino;

wherein R is

(a) isopropyl,

(b) isobutyl,

25 (c) phenylmethyl, or

(d) C_3-C_7 cycloalkyl;

wherein R_1 is

(a) hydrogen,

(b) C_1-C_3 alkyl,

30 (c) aryl,

(d) C_3-C_7 cycloalkyl,

(e) -Het,

(f) C_1-C_3 alkoxy, or

(g) C_1-C_3 alkylthio;

35 wherein R_2 is

(a) hydrogen, or

(b) $-CH(R_3)R_4$;

wherein R_3 is

-4-

- 5 (a) hydrogen,
(b) hydroxy,
(c) C₁-C₅ alkyl,
(d) C₃-C₇ cycloalkyl,
(e) aryl,
(f) -Het,
(g) C₁-C₃ alkoxy, or
(h) C₁-C₃ alkylthio;

wherein R₄ at each occurrence is the same or different and is

- 10 (a) hydrogen, or
(b) C₁-C₅ alkyl;

wherein R₅ is

- 15 (a) C₁-C₆ alkyl,
(b) C₃-C₇ cycloalkyl,
(c) aryl,
(d) -Het, or
(e) 5-oxo-2-pyrrolidinyl;

wherein R₆ is

- 20 (a) -S-aryl,
(b) -S-C₃-C₇ cycloalkyl, or
(c) -S-C₁-C₆ alkyl;

wherein R₇ is

- 25 (a) hydrogen,
(b) C₁-C₅ alkyl,
(c) hydroxy,
(d) amino C₁-C₄ alkyl-,
(e) guanidiny C₁-C₃ alkyl-,
(f) aryl,
(g) -Het,
30 (h) methylthio,
(i) C₃-C₇ cycloalkyl, or
(j) amino;

wherein R₈ is

- 35 (a) hydrogen,
(b) C₁-C₅ alkyl,
(c) hydroxy,
(d) aryl,
() -Het,

-5-

(f) guanidiny1 C₁-C₃ alkyl-, r(g) C₃-C₇ cycloalkyl;wherein R₉ is

(a) hydrogen,

5 (b) hydroxy,

(c) amino C₁-C₄ alkyl-, or(d) guanidiny1 C₁-C₃ alkyl-;wherein R₁₀ is

(a) hydrogen,

10 (b) C₁-C₅ alkyl,(c) -(CH₂)_n R₁₆,(d) -(CH₂)_n R₁₇,(e) C₃-C₇ cycloalkyl,

(f) a pharmaceutically acceptable cation,

15 (g) -(CHR₂₃)-CH₂-R₁₅, or(h) -CH₂-(CHR₁₂)-R₁₅;wherein R₁₁ is -R or -R₂;wherein R₁₂ is -(CH₂)_n-R₁₃;wherein R₁₃ is

20 (a) aryl,

(b) amino,

(c) mono-, di or tri-C₁-C₃ alkylamino,

(d) -Het,

(e) C₁-C₅ alkyl25 (f) C₃-C₇ cycloalkyl,(g) C₁-C₅ alkenyl,(h) C₃-C₇ cycloalkenyl,

(i) hydroxy,

(j) C₁-C₃ alkoxy,30 (k) C₁-C₃ alkanoyloxy,

(l) mercapto,

(m) C₁-C₃ alkylthio,

(n) -COOH,

(o) -CO-O-C₁-C₆ alkyl,35 (p) -CO-O-CH₂-(C₁-C₃ alkyl)-N(C₁-C₃ alkyl)₂,(q) -CO-NR₂₂R₂₆;(r) C₄-C₇ cyclic amino,(s) C₄-C₇ cycl alkylamino,

-6-

- (t) guanidyl,
- (u) yano,
- (v) N-cyanoguanidyl,
- (w) cyanoamino,
- 5 (x) (hydroxy C_2-C_4 alkyl)amino, or
- (y) di-(hydroxy C_2-C_4 alkyl)amino;

wherein R_{14} is

- (a) hydrogen,
- (b) C_1-C_{10} alkyl,
- 10 (c) $-(CH_2)_n-R_{18}$,
- (d) $-(CH_2)_n-R_{19}$,
- (e) $-(CHR_{25})-CH_2-R_{15}$,
- (f) $-CH_2-(CHR_{12})-R_{15}$,
- (g) (hydroxy C_1-C_8 alkyl), or
- 15 (h) $(C_1-C_3$ alkoxy) C_1-C_8 alkyl;

wherein R_{15} is

- (a) hydroxy,
- (b) C_3-C_7 cycloalkyl,
- (c) aryl,
- 20 (d) amino,
- (e) mono-, di-, or tri- C_1-C_3 alkylamino,
- (f) mono- or di-[hydroxy C_2-C_4 alkyl]amino,
- (g) -Het,
- (h) C_1-C_3 alkoxy-,
- 25 (i) C_1-C_3 alkanoyloxy-,
- (j) mercapto,
- (k) C_1-C_3 alkylthio-,
- (l) C_1-C_3 alkyl,
- (m) C_4-C_7 cyclic amino,
- 30 (n) C_4-C_7 cycloalkylamino,
- (o) C_1-C_3 alkenyloxy,
- (p) C_3-C_7 cycloalkenyl;

wherein R_{16} is

- (a) aryl,
- 35 (b) amino,
- (c) mono- or di- C_1-C_3 alkylamino,
- (d) hydroxy,
- (e) C_3-C_7 cycloalkyl,

-7-

(f) C_4-C_7 cyclic amin , r(g) C_1-C_3 alkanoyl xy;wherein R_{17} is

(a) -Het,

5 (b) C_1-C_3 alkenyl,(c) C_3-C_7 cycloalkenyl,(d) C_1-C_3 alkoxy,

(e) mercapto,

(f) C_1-C_3 alkylthio,

10 (g) -COOH,

(h) -CO-O- C_1-C_6 alkyl,(i) -CO-O- $CH_2-(C_1-C_3$ alkyl)- $N(C_1-C_3$ alkyl) $_2$,(j) -CO-NR $_{22}$ R $_{26}$,(k) tri- C_1-C_3 alkylamino,

15 (l) guanidyl,

(m) cyano,

(n) N-cyanoguanidyl,

(o) (hydroxy C_2-C_4 alkyl)amino, or(p) di-(hydroxy C_2-C_4 alkyl)amino;20 wherein R_{18} is

(a) amino,

(b) mono-, or di- C_1-C_3 alkylamino, or(c) C_4-C_7 cyclic amino;wherein R_{19} is

25 (a) aryl,

(b) -Het,

(c) tri- C_1-C_3 alkylamino,(d) C_3-C_7 cycloalkyl,(e) C_1-C_3 alkenyl,30 (f) C_3-C_7 cycloalkenyl,

(g) hydroxy,

(h) C_1-C_3 alkoxy,(i) C_1-C_3 alkanoyloxy,

(j) mercapto,

35 (k) C_1-C_3 alkylthio,

(l) -COOH,

(m) -CO-O- C_1-C_6 alkyl,(n) -CO-O- $CH_2-(C_1-C_3$ alkyl)- $N(C_1-C_3$ alkyl) $_2$,

-8-

- (o) $-\text{CO}-\text{NR}_{22}\text{R}_{26}$,
- (p) C_4-C_7 cycloalkylamin ,
- (q) guanidyl,
- (r) cyano,
- 5 (s) N-cyanoguanidyl,
- (t) cyanoamino,
- (u) (hydroxy C_2-C_4 alkyl)amino,
- (v) di-(hydroxy C_2-C_4 alkyl)amino; or
- (w) $-\text{SO}_3\text{H}$;

10 wherein R_{20} is

- (a) hydrogen,
- (b) C_1-C_5 alkyl, or
- (c) aryl- C_1-C_5 alkyl;

wherein R_{21} is

- 15 (a) $-\text{NH}_2$, or
- (b) $-\text{OH}$;

wherein R_{22} is

- (a) hydrogen, or
- (b) C_1-C_3 alkyl;

20 wherein R_{23} is

- (a) $-(\text{CH}_2)_n-\text{OH}$,
- (b) $-(\text{CH}_2)_n-\text{NH}_2$,
- (c) aryl, or
- (d) C_1-C_3 alkyl;

25 wherein R_{24} is

- (a) $-\text{R}_1$,
- (b) $-(\text{CH}_2)_n-\text{OH}$, or
- (c) $-(\text{CH}_2)_n-\text{NH}_2$;

wherein R_{25} is $-(\text{CH}_2)_n-\text{R}_{13}$;30 wherein R_{26} is

- (a) hydrogen,
- (b) C_1-C_3 alkyl, or
- (c) phenyl- C_1-C_3 alkyl;

wherein i is one to three, inclusive;

35 wherein m is one or two;

wherein for each occurrence n is independently an integer of
zero to five, inclusive;

wherein p is zero to 2 inclusive;

-9-

wherein q is 1 to 5, inclusive;

wherein Q is

- (a) $-\text{CH}_2-$,
- (b) $-\text{CH}(\text{OH})-$,
- (c) $-\text{O}-$, or
- (d) $-\text{S}-$; and

wherein M is

- (a) $-\text{CO}-$, or
- (b) $-\text{CH}_2-$;

wherein aryl is phenyl or naphthyl substituted by zero to 3 to the following:

- (a) C_1-C_3 alkyl,
- (b) hydroxy,
- (c) C_1-C_3 alkoxy,
- (d) halo,
- (e) amino,
- (f) mono- or di- C_1-C_3 alkylamino,
- (g) $-\text{CHO}$,
- (h) $-\text{COOH}$,
- (i) COOR_{26} ,
- (j) CONHR_{26} ,
- (k) nitro,
- (l) mercapto,
- (m) C_1-C_3 alkylthio,
- (n) C_1-C_3 alkylsulfinyl,
- (o) C_1-C_3 alkylsulfonyl,
- (p) $-\text{N}(\text{R}_4)-\text{C}_1-\text{C}_3$ alkylsulfonyl,
- (q) SO_3H ,
- (r) SO_2NH_2 ,
- (s) $-\text{CN}$, or
- (t) $-\text{CH}_2\text{NH}_2$;

wherein -Het is a 5- or 6-membered saturated or unsaturated ring containing from one to three heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur; and including any bicyclic group in which any of the above heterocyclic rings is fused to a benzene ring, which heterocyclic moiety is substituted with zero to 3 of the following:

- (i) C_1-C_6 alkyl,

-10-

- (ii) hydroxy,
- (iii) trifluor methyl,
- (iv) C_1-C_4 alkyl,
- (v) halo,
- (vi) aryl,
- (vii) aryl C_1-C_4 alkyl-,
- (viii) amino, and
- (ix) mono- or di- C_1-C_4 alkylamino;

with the overall provisos that

- (1) X is $R_6-(CH_2)_1-C(=O)-$ only when A_8 , B_7 , and C_8 are absent;
- (2) R_{18} or R_{19} is hydroxy, mercapto, or amino, or a mono-substituted nitrogen containing group bonded through the nitrogen only when n is not one;
- (3) R_{12} is $-(CH_2)_n-R_{13}$ and n is zero and both R_{13} and R_{15} are oxygen-, nitrogen-, or sulfur-containing substituents bonded through the hetero atom, only when the hetero atom is not also bonded to hydrogen;
- (4) R_{25} is $-(CH_2)_n-R_{13}$ and n is zero only when R_{13} is other than a primary or secondary nitrogen-containing group hydroxy or mercapto group or when R_4 of $-N(R_4)R_{14}$ is other than hydrogen; and
- (5) R_{17} or R_{19} is $-COOH$ only when n for that moiety is other than zero;
- (6) R_{16} or R_{17} is an amino-containing substituent, hydroxy, mercapto, or -Het bonded through the hetero atom only when n for that substituent is an integer from two to five, inclusive; and
- (7) when R_{12} is $-(CH_2)_n-R_{13}$ and n is zero, then R_{13} and R_{15} cannot both be $-COOH$;

or a carboxy-, amino-, or other reactive group-protected form
or a pharmaceutically acceptable acid addition salt thereof.

These compounds are shown in relation to the human renin substrate as follows:

| | | | | | | | | |
|---|-------|-------|-------|-------|----------|----------|----------|----------------------|
| | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| | -His | Pro | Phe | His | Leu | Val | Ile | His- |
| X | A_6 | B_7 | C_8 | D_9 | E_{10} | F_{11} | G_{12} | H_{13} I_{14} Z, |

The present invention provides peptide inhibitors of renin which contain modification of the Phe⁸ (angiotensinogen numbering) position. These modifications involve the insertion of a heteroatom-containing fragment into the side chain of the residue occupying the

-11-

Phe⁸ position. These changes in the normal Phe side chain result in dramatic changes in the overall length and size of the side chain. The hetero-atom also alters the polarity, angles, and H-bonding ability of the side chain elements. Additionally, changes in the backbone may be made such as substitution of oxygen for nitrogen in the corresponding α -amino acid. These changes, in addition to being chemically novel to renin inhibitors, also confer stability to the position 8-9 bond to the action of chymotrypsin and elastase due to the lack of fit of the lengthened side chain into the active site pocket of these enzymes.

Examples of pharmaceutically acceptable acid addition salts include: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, palmoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate.

The carbon atom content of various hydrocarbon-containing moieties is indicated by a prefix designating the minimum and maximum number of carbon atoms in the moiety, i.e., the prefix (C₁-C_j) indicates a moiety of the integer "i" to the integer "j" carbon atoms, inclusive. Thus (C₁-C₄)alkyl refers to alkyl of one to 4 carbon atoms, inclusive, or methyl, ethyl, propyl, butyl, and isomeric forms thereof. C₄-C₇cyclic amino indicates a monocyclic group containing one nitrogen and 4 to 7 carbon atoms.

Examples of (C₃-C₁₀)cycloalkyl which include alkyl-substituted cycloalkyl, are cyclopropyl, 2-methylcyclopropyl, 2,2-dimethylcyclopropyl, 2,3-diethylcyclopropyl, 2-butylcyclopropyl, cyclobutyl, 2-methylcyclobutyl, 3-propylcyclobutyl, cyclopentyl, 2,2-dimethylcyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl and isomeric forms thereof.

Examples of aryl include phenyl, naphthyl, (o-, m-, p-)tolyl, (o-, m-, p-)ethylphenyl, 2-ethyl-tolyl, 4-ethyl-o-tolyl, 5-ethyl-m-tolyl, (o-, m-, or p-)propylphenyl, 2-propyl-(o-, m-, or p-)tolyl, 4-isopropyl-2,6-xylyl, 3-propyl-4-ethylphenyl, (2,3,4- 2,3,6-, or

2,4,5-)trimethylphenyl, (o-, m-, or p-)fluorophenyl, (o-, m-, or p-trifluoromethyl)phenyl, 4-fluoro-2,5-xylyl, (2,4-, 2,5-, 2,6-, 3,4-, or 3,5-)difluorophenyl, (-, m-, or p-)chlorophenyl, 2-chloro-p-tolyl, (3-, 4-, 5- or 6-)chloro-o-tolyl, 4-chloro-2-propylphenyl, 2-isopropyl-4-chlorophenyl, 4-chloro-3-fluorophenyl, (3- or 4-)chloro-2-fluorophenyl, (o-, m-, or p-)trifluoro-methylphenyl, (o-, m-, or p-)ethoxyphenyl, (4- or 5-)chloro-2-methoxy-phenyl, and 2,4-dichloro-(5- or 6-)methylphenyl.

Examples of -Het include: 2-, 3-, or 4-pyridyl, imidazolyl, indolyl, N^{1a}-formyl-indolyl, N^{1a}-C₂-C₃alkyl-C(=O)-indolyl, [1,2,4]-triazolyl, 2-, 4-, or 5-pyrimidinyl, 2- or 3-thienyl, piperidinyl, pyrrolyl, pyrrolinyl, pyrrolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, pyrazinyl, piperazinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, quinolinyl, isoquinolinyl, benzimidazolyl, benzothiazolyl, benzoxazolyl, furyl, thienyl, and benzothienyl. Each of these moieties may be substituted as noted above.

As would be generally recognized by those skilled in the art of organic chemistry, a heterocycle as defined herein for -Het would not be bonded through oxygen or sulfur or through nitrogen which is within a ring and part of a double bond.

Halo is halogen (fluoro, chloro, bromo, or iodo) or trifluoromethyl.

Examples of pharmaceutically acceptable cations include: pharmacologically acceptable metal cations, ammonium, amine cations, or quaternary ammonium cations. Especially preferred metal cations are those derived from the alkali metals, e.g., lithium, sodium, and potassium, and from the alkaline earth metals, e.g., magnesium and calcium, although cationic forms of other metals, e.g., aluminum, zinc, and iron are also within the scope of this invention. Pharmacologically acceptable amine cations are those derived from primary, secondary, or tertiary amines.

The novel peptides herein contain both natural and synthetic amino acid residues. These residues are depicted using standard amino acid abbreviations (see, e.g., Roberts, et al., Basic Principles of Organic Chemistry, pp. 703-705 (New York 1965)) unless otherwise indicated.

-13-

All the renin-inhibiting compounds of the present invention may be administered in the conventional forms, such as disclosed in U.S. Patent No. 4,424,207 which is incorporated by reference herein. Likewise, the amounts disclosed in the U.S. Patent No. 4,424,207 are examples applicable to the compounds of the present invention.

Preferably, the dosages of the present invention are for oral administration for treatment of humans to effect renin inhibition for the purpose of favorably affecting blood pressure. For this purpose, the compounds are administered from 0.1 mg to 1000 mg per kg per dose, administered from 1 to 4 times daily. Equivalent dosages for other routes of administration are also employed.

The exact dose depends on the age, weight, and condition of the patient and on the frequency and route of administration. Such variations are within the skill of the practitioner or can readily be determined.

The compounds of the present invention may be pharmaceutically acceptable salts both those which can be produced from the free bases by methods well known in the art and those with which acids have pharmacologically acceptable conjugate bases.

The compounds of the present invention are preferably orally administered in the form of pharmacologically acceptable acid addition salts. Preferred pharmacologically acceptable salts for oral administration include the citrate and aspartate salts, although any pharmacologically acceptable salt is useful in this invention, including those listed above. These salts may be in hydrated form.

The compounds of the present invention are prepared as depicted in the charts and as described more fully in the Preparations and Examples.

The description below refers to the structures depicted on the formula page and in the Charts A-E. In those formulas, all variables are as defined above except where noted.

Generally, the renin inhibiting polypeptides may be prepared by either polymer assisted or solution phase peptide synthetic procedures analogous to those described hereinafter or to those methods known in the art. For example, the carboxylic moiety of N^ε-t-butyloxycarbonyl (Boc)-substituted amino acid derivatives having suitable side chain protecting groups, if necessary, may be condensed with the amino functionality of a suitably protected amino acid, peptide or

-14-

polymer-bound peptide using a conventional coupling protocol such as dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) in methylene chloride or dimethylformamide. The synthetic procedures used to incorporate the novel moieties herein are also described, for example, in U.S. patents 4,424,207; 4,470,971; 4,477,440; 4,477,441; 4,478,826; 4,478,827; 4,479,941; and 4,485,099, and copending application Serial No. 753,198, filed 9 July 1985, all of which are expressly incorporated by reference herein. See, also, published European patent applications 45,161; 45,665; 53,017; 77,028; 77,029; 81,783; 104,041; 111,266; 114,993; and 118,223.

Following coupling reaction completion, the N^{α} -Boc moiety may be selectively removed with 45% trifluoroacetic acid/2% anisole (v/v) in methylene chloride. Neutralization of the resultant trifluoroacetate salt may be accomplished with 10% diisopropylethylamine in methylene chloride. In the case of polymer-assisted peptide synthesis, this stepwise, coupling strategy may be partially or completely automated to provide the desired peptide-polymer intermediates. Anhydrous hydrofluoric acid treatment of the peptide-polymer intermediate may then be used to effect simultaneous protecting group removal and cleavage of the peptide from its polymeric support. A notable exception to this includes $N^{1\alpha}$ -formyl-indolyl-substituted peptides in which the $N^{1\alpha}$ -formyl-indolyl moiety is stable to TFA or HF but may be removed by NH_3 or NaOH. Because FTrp is somewhat unstable to base in synthetic procedures, possibly causing lower yields, it may be desirable in solution phase synthesis to introduce the FTrp-containing moiety late in the synthetic sequence so that it is not exposed to such conditions.

The incorporation of $N^{1\alpha}$ -formyl-Trp into compounds of the present invention is easily accomplished because of the commercial availability of N^{α} -Boc- $N^{1\alpha}$ -formyl-Trp-OH. However, the $N^{1\alpha}$ -formyl moiety may be introduced into indolyl-substituted amino acid derivatives or related compounds by reaction with HCl-formic acid as reported in the literature, see A. Previero et al, *Biochim. Biophys. Acta* 147, 453 (1967); Y.C.S. Yang et al, *Int. J. Peptide Protein Res.* 15, 130 (1980).

Generally, methods of alkylation useful in alkylating histidine for use in the present invention are found in Cheung, S.T. et al, *Can. J. Chem.*, Vol 55, pp. 906-910 (1977). However it is now found

-15-

that in Cheung, S. T. et al, methods it is critical that the reaction conditions for the alkylation of histidine be anhydrous. Further, it is now found also that during work-up instead of adding water directly to the reaction mixture, it is preferred that a buffered aqueous solution be added to the reaction mixture, for example, aqueous sodium or potassium hydrogen sulfate.

This is analogous to that found in Cheung, S.T. et al, Can. J. Chem. Vol 55, pp. 906-910 (1977)), however, it has been found that the process requires anhydrous reaction conditions which are not taught by Cheung et al.

Variations in the above description for starting materials, reactants, reaction conditions and required protecting groups to obtain other such N-alkylated compounds are known to an ordinarily skilled chemist or are readily available in the literature.

These peptides may also be prepared by the standard solid phase techniques of Merrifield. Appropriate protecting groups, reagents, and solvents for both the solution and solid phase methods can be found in "The Peptides: Analysis, Synthesis, and Biology," Vols. 1-5, eds. E. Gross and T. Meienhofer, Academic Press, NY, 1979-1983.

The compounds of the present invention may be in either free form or in protected form at one or more of the remaining (not previously protected) peptide, carboxyl, amino, hydroxy, or other reactive groups. The protecting groups may be any of those known in the polypeptide art. Examples of nitrogen and oxygen protection groups are set forth in T.W. Greene, Protecting Groups in Organic Synthesis, Wiley, New York, (1981); J.F.W. McOmie, ed. Protective Groups in Organic Chemistry, Plenum Press (1973); and J. Fuhrhop and G. Benzlin, Organic Synthesis, Verlag Chemie (1983). Included among the nitrogen protective groups are t-butoxycarbonyl (Boc), benzyloxycarbonyl, acetyl, allyl, phthalyl, benzyl, benzoyl, trityl and the like.

In general, the processes of the present invention may be carried out in the following manner.

The synthesis of the key intermediate, Boc-(R,S)-3-(phenylthio)alanine, is shown in Chart A. This synthesis follows the method of G. Glynn and D. Beight, Tet. Lett.; 25:2655 (1984). Boc-Serine (Boc-D,L-Ser may be used as chirality is lost at a later step) is

-16-

converted to its methyl ester using a five-fold excess of methyl iodide and two equivalents of NaHCO_3 in DMF as solvent. The alcohol (A-2) is then converted to the mesylate with methanesulfonyl chloride and 1 equiv. of Et_3N in CH_2Cl_2 . Addition of a second equivalent of Et_3N gives the elimination product A-3. Compound A-3 is stirred with 1.1 equivalents of thiophenol and a catalytic amount of Et_3N in MeOH to give A-4. Hydrolysis of the methyl ester with aq. NaOH in MeOH, followed by acidification, gives Boc-(R,S)-3-(phenylthio)alanine A-5.

The peptides are synthesized by methods known to the art. See, for instance, the series "The Peptides: Analysis, Synthesis, Biology, Vols. 1-5, eds. E. Gross and J. Meienhofer, Academic Press, New York, 1979-1983. The assembly of Boc-(R,S)-3-(phenylthio)alanyl-His-Leu Ψ [CH(OH)CH₂]Val-Ile-2-(amidomethyl)pyridine is shown in Chart B. Boc-Ile and 2-(aminomethyl)pyridine (both commercially available) are coupled using DCC in CH_2Cl_2 and aq. NaHCO_3 to give B-3. Boc-Leu Ψ [CH(OTBDMS)-CH₂]Val-OH is coupled to Ile-AMP (AMP-2-(amidomethyl)pyridine) using DEPC (diethyl cyanophosphonate) and Et_3N in CH_2Cl_2 to give B-4. The Boc group of B-4 is removed with TFA and CH_2Cl_2 and the resulting TFA salt is neutralized by extraction with CH_2Cl_2 and aq. NaHCO_3 . Leu Ψ [CH(OTBDMS)-CH₂]Val-Ile-AMP is coupled with Boc-His(Tos)-OH using DEPC and Et_3N in CH_2Cl_2 to give B-5. The Boc group and tert-butyldimethylsilyl group of B-7 are removed with TFA and CH_2Cl_2 . The resulting TFA salt is neutralized via extraction with CH_2Cl_2 and aq. NaHCO_3 to give B-6. Compound B-6 is coupled with Boc-(R,S)-3-(phenylthio)alanine using DCC in CH_2Cl_2 . The tosyl protecting group on the histidine side chain is removed with 1-HOBT and MeOH to give the final product, B-7. In a similar manner, the mercaptide anion of cycloalkyl-SH or alkyl-SH (formed from, for instance, the commercially available mercaptan and NaOMe or NaH) is added to A-3 and carried through the sequences of Charts A and B to give compounds such as A-5 and B-7 in which cycloalkyl-S- and alkyl-S-replace aryl-S-.

The syntheses of key intermediates C-4A and C-5 are shown in Chart C. Commercial L-malic acid is converted to the halide C-2 via the method of Larcheveque and Petit, Tet. Letters, 25:3705 (1984) and references cited therein. Reaction of C-2 with thiophenoxide anion (generated from thiophenol and base) gives ester C-3. Ester C-3 is hydrolyzed with aqueous NaOH in MeOH, followed by acidification, to

-17-

giving acid C-3A. Acid C-3A is converted to the O-acetate with Ac_2O in pyridine to give C-4A. Coupling of C-3A with $\text{His}(\text{Tos})\text{-LeuY-}[\text{CH}(\text{OH})\text{CH}_2]\text{Val-Ile-AMP}$ using DEPC and Et_3N in CH_2Cl_2 , followed by removal of the tosyl group with 1-HOBT, gives C-5A. Compound C-3 is converted to the carbamate derivative C-4 with diethyl carbamoyl chloride, NaH , Et_3N , and THF. Compound C-4 is hydrolyzed to the acid with aqueous NaOH in MeOH , followed by acidification. Coupling of C-5 with $\text{His}(\text{Tos})\text{-LeuY}[\text{CH}(\text{OH})\text{CH}_2]\text{Val-Ile-AMP}$ using DEPC and Et_3N in CH_2Cl_2 , followed by removal of the tosyl group with 1-HOBT in MeOH , gives C-6. In a similar manner, the mercaptide anions, cycloalkyl- S^- and alkyl- S^- (from the mercaptan and a base such as NaOMe and NaH), may be added to C-2 and carried through the above sequence to give compounds of the general structures C-5A and C-6 with cycloalkyl- S^- and alkyl- S^- replacing aryl- S^- .

The synthesis of D-5 is outlined in Chart D. Reaction of thiophenoxide anion with ethyl acrylate gives ester D-2. Hydrolysis of D-2 with aqueous NaOH and MeOH , followed by acidification, gives acid D-3. Coupling of D-3 with $\text{His}(\text{Tos})\text{-LeuY}[\text{CH}(\text{OH})\text{CH}_2]\text{Val-Ile-AMP}$ using DEPC and Et_3N in CH_2Cl_2 , followed by removal of the tosyl group with 1-HOBT in MeOH , gives D-5.

Chart E outlines the synthesis of intermediates for the preparation of compounds of this invention wherein X is $\text{R}_6\text{-(CH}_2)_1\text{-C(=O)-}$ from known (commercially available) halo ester E-1 or halo ester E-3. Halo ester E-1 is reacted with the mercaptide anion of R_6SH to give thio ether E-2, which is hydrolyzed to give acid E-5. Alternatively halo acid E-3 is converted to a salt such as the sodium salt E-4 which is reacted with the anion of R_6SH followed by acidification to give acid E-5. In the same manner as the conversion of D-3 to D-5, E-5 may be incorporated into peptides of this invention, e.g., D-5.

30 DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following Preparations and Examples illustrate the present invention.

In the Preparations and Examples below and throughout this document: DMF is dimethylformamide,

35 MsCl is methanesulfonyl chloride,

Et_3N is triethylamine,

CH_2Cl_2 is methylene chloride,

MeOH is methanol,

-18-

TFA is trifluoroacetic acid,
DEPC is diethylcyanophosphonate,
1-HOBT is 1-hydroxybenzotriazole,
TBDMS is tert-butyldimethylsilyl,

5 Tos is p-toluenesulfonyl,

His(Tos) is histidyl protected on the imidazole nitrogen with Tos,

Ac₂O is acetic anhydride,

pyr is pyridine,

THF is tetrahydrofuran,

10 AMP is (2-pyridinyl)methylamino,

DCC is dicyclohexylcarbodiimide,

NaHCO₃ is sodium bicarbonate, and

Na₂SO₄ is sodium sulfate.

Preparation 1 Boc-Serine Methyl Ester

15 Chart A (A-1 to A-2).

A mixture of 2.05 g (0.010 moles) of Boc-L-Ser, 3.27 ml (0.050 moles) in MeI, 1.68 g (0.020 moles) of NaHCO₃, and 25 ml of DMF is stirred overnight at room temperature. DMF is removed in vacuo and the residue is extracted with EtOAc, H₂O, and brine. The organic
20 layers are filtered through Na₂SO₄, concentrated, and chromatographed on silica gel using 2% MeOH-CH₂Cl₂ to give 2.03 g (93%) of titled product.

TLC (4% MeOH-CH₂Cl₂): R_f is 0.41.

¹H-NMR (CDCl₃): δ 1.45, s, 9H; 2.52, t, 1 H; 3.78, s, 3H; 3.91, m,

25 2H; 4.30, m, 1 H; 5.45, bd, 1 H.

Preparation 2 Boc-(R,S)-3-(Phenylthio)alanine Methyl Ester

Chart A (A-2 to A-3 to A-4).

To Boc-Ser-OCH₃ (0.099 g, 0.451 mmoles) and Et₃N (0.069 ml, 0.497 mmoles) in 3 ml of CH₂Cl₂ at 0° is added 0.038 ml (0.497
30 mmoles) of methanesulfonyl chloride. After 2 1/3 hours, an additional 0.07 ml (0.5 mmoles) of Et₃N is added and the ice bath is removed. After stirring an additional 1 2/3 hours, the reaction mixture is extracted with CH₂Cl₂, H₂O, and brine. The organic layers are filtered through Na₂SO₄, concentrated and chromatographed on silica
35 gel to give 0.0723 g (80%) of methylene intermediate A-3 (R_f is 0.61, 20% EtOAc-hexane).

A solution of 0.0723 g (0.358 mmoles) of the methylene intermediate, 0.043 g (0.393 mmoles) of thiophenol, 4 drops of Et₃N, and

-19-

15 ml of MeOH is stirred overnight. MeOH is then removed in vacuo and the residue kept under high vacuum for several hours to give 0.1096 g (98%) of titled product.

TLC (20% EtOAc-hexane): R_f is 0.38.

5 ¹H-NMR (CDCl₃): δ 1.41, s, 9 H; 3.36, d, 2H; 3.54, s, 3 H; 4.50, m, 1 H; 5.25, bd, 1H; 7.3, m, 5H.

Preparation 3 Boc-(R,S)-3-(Phenylthio)alanine

Chart A (A-4 to A-5)

To a solution of 0.100 g (0.321 mmoles) of the ester of Preparation 2 in 3-4 ml of MeOH is added dropwise 0.48 ml of 1 N NaOH (exotherm). After stirring for 1 hour, MeOH is removed in vacuo and the residue is extracted with CH₂Cl₂ and 2N NaCl. The organic layers are filtered through Na₂SO₄ and taken to dryness in vacuo to give 0.0828 g (87%) of product. Crystallization from CH₂Cl₂-hexane gives 15 0.07 g of product, mp. 104-106°.

TLC (4% MeOH-CH₂Cl₂-HOAc): R_f is 0.30.

Mass spec.: m/z at 297 (theory, 297).

Calcd for C₁₄H₁₈NO₄S: C, 56.54; H, 6.44; N, 4.71. Found: C, 56.40; H, 6.58; N, 4.60.

20 ¹H-NMR (CDCl₃): δ 1.42, s; 1.35, m; 1.4, m; 5.45, bd; 7.3, m.

Preparation 5 Boc-Ile-AMP

Chart B (B-1 + B-2 to B-3).

To 3.15 g (0.0136 moles) of Boc-Ile and 2.08 g (0.0136 moles) of 1-HOBT in 50 ml of CH₂Cl₂ is added 1.34 g (0.0123 moles) of 2-(aminomethyl)pyridine. After 5-10 minutes, 2.81 g (0.0136 moles) of DCC is added. After 35 minutes, DCU is filtered off and the filtrate is washed with aqueous NaHCO₃. The organic layers are filtered through Na₂SO₄, concentrated, re-filtered to remove DCU, and chromatographed on silica gel using 4% MeOH/CH₂Cl₂ to give 3.94 g (100%) of product. 25 The solid is crystallized from EtOAc/hexane to give the titled product: 2.28 g of a first crop, m.p. 102-103.5° C, and 1.50 g of second and third crops.

Calcd. for C₁₇N₂N₃O₃: C, 63.52; H, 8.47; N, 13.07. Found: C, 63.37; H, 8.55; N, 12.86.

35 ¹H-NMR (CDCl₃): δ 0.89-0.97, m; 1.43, s, Boc; 1.76, s; 4.0, m, α-CH; 4.56, d (J=5.1), CH₂Py; 5.05, d, NH; 7.0-8.5, m, aromatic.

Preparation 6 Boc-LeuV[CH(OH)CH₂]Val-Ile-AMP

B-3 to B-4.

-20-

Boc-Ile-AMP (1.60 g, 4.98 mmol) and 50% TFA-CH₂Cl₂ (20 ml) is stirred for 30 minutes, after which TFA and CH₂Cl₂ are removed in vacuo. The residue is exhaustively extracted with CH₂Cl₂ and aq. NaHCO₃. The organic layers are filtered through Na₂SO₄ and taken to dryness.

To a solution of 0.0509 g (0.114 mmol) of Boc-LeuW[CH(OTBDMS)-CH₂]Val-OH, 0.0253 g (0.114 mmol) of Ile-AMP, and 0.0154 g (0.114 mmol) of 1-HOBT in 3 ml of CH₂Cl₂ is added 0.0235 g (0.114 mmol) of DCC. After stirring over the weekend (reaction over much sooner), DCU is filtered off and the filtrate is extracted with CH₂Cl₂ and aq. NaHCO₃. The organic layers are filtered through Na₂SO₄, concentrated, and the crude product is chromatographed on silica gel using 3% MeOH-CH₂Cl₂ (NH₄OH sat'd) to give 0.0630 g (85%) of product.

TLC (4% MeOH-CH₂Cl₂): R_f is 0.14.

¹H-NMR (CDCl₃): δ 0.11, s; 0.90, s; 0.83-0.95, m; 1.43, s; 3.1, m; 4.5, m; 6.10, bd; 7.1-7.8, m; 9.03, bd.

Preparation 7 Boc-His(Tos)-LeuW[CH(OTBDMS)CH₂]Val-Ile-AMP

B-4 to B-5.

A solution of 0.063 g (0.097 mmol) of B-4 from Preparation 6 in 5 ml of TFA-CH₂Cl₂ (1:1) is stirred for 15 minutes at room temperature and then concentrated in vacuo. The residue is extracted with CH₂Cl₂ and aq. NaHCO₃ and the organic layers are filtered through Na₂SO₄ and concentrated to dryness. To this is added Boc-His(Tos)-OH (0.06 g, 0.146 mmol) and 4 ml of CH₂Cl₂, followed by 0.030 g (0.146 mmol) of DCC. After stirring 2.5 hours at room temperature, DCU is filtered off and the filtrate is concentrated. EtOAc is added and DCU is filtered off again. The filtrate is concentrated and the residue is chromatographed on silica gel using 2% MeOH-CH₂Cl₂ to 4% MeOH-CH₂Cl₂ (NH₄OH sat'd) to give 0.0793 g (87%) of the titled product.

¹H-NMR (CDCl₃): δ 0.07, s; 0.88-0.95, m; 1.40, s; 2.42, s; 5.95, bd; 6.20, bd; 6.65, bd; 7.05-8.5, m.

TLC (4% MeOH-CH₂Cl₂-NH₄OH sat'd): R_f is 0.38.

Preparation 8 His(Tos)-LeuW[CH(OH)CH₂]Val-Ile-AMP

B-5 to B-6.

A solution of 0.079 g (0.084 mmol) of B-5 from Preparation 7 in 10 ml of TFA-CH₂Cl₂ (1:1) is stirred for 4 hours at room temperature. TFA and CH₂Cl₂ are then removed in vacuo and the residue is

-21-

extracted with CH_2Cl_2 and aq NaHCO_3 . The organic layers are filtered through Na_2SO_4 and concentrated. The crude product is chromatographed on silica gel (10 g) using 6% $\text{MeOH}-\text{CH}_2\text{Cl}_2$ (NH_4OH sat'd) to give 0.0513 g (84%) of the titled product.

5 TLC (8% $\text{MeOH}-\text{CH}_2\text{Cl}_2-\text{NH}_4\text{OH}$): R_f is 0.40.

Example 1 Boc-((S-phenyl)-DL-Cys)-His-Leu $\text{W}[\text{CH}(\text{OH})\text{CH}_2]$ Val-Ile-AMP
B-6 to B-7.

To 0.0056 g (0.0187 mmoles) of Boc-(R,S)-3(phenylthio)alanine (A-5 from Preparation 3) and 0.0091 g (0.0125 mmoles) of B-6 from
10 Preparation 8 in 3 ml of CH_2Cl_2 is added 0.0039 g (0.0187 mmoles) of DCC. After stirring for 30 minutes, the reaction mixture is chromatographed on silica gel (10 g) using 6% $\text{MeOH}-\text{CH}_2\text{Cl}_2$ (NH_4OH sat'd) to give 12.7 mg of the (impure) Tos-protected titled product (R_f is 0.42 in 8% $\text{MeOH}-\text{CH}_2\text{Cl}_2-\text{NH}_4\text{OH}$). This Tos-protected material is stirred for
15 2 days with 0.02 g of 1-HOBT in 1 ml of MeOH, after which MeOH is removed in vacuo. The residue is chromatographed on silica gel (10 g) using 6% $\text{MeOH}-\text{CH}_2\text{Cl}_2$ (NH_4OH sat'd) to give 0.0077 g of the titled product.

TLC (6% $\text{MeOH}-\text{CH}_2\text{Cl}_2-\text{NH}_4\text{OH}$): R_f is 0.11.

20 FAB mass spec.: $[M+H]$ at m/z 851 (calcd 851).

$^1\text{H-NMR}$ (CDCl_3) for the Tos-protected titled product: δ 1.40, s; 1.43, s (Boc group of each isomer); 2.42 bs (tosyl CH_3).

HPLC: Brownlee C_{18} column, phosphate pH 3 buffer: 50% A-50% B, k' is 8.3 (broad), 94.3% purity; flow rate 1.5 ml/min, A 0.1, 225 nM.

25 Preparation 9 Ethyl 3-(Phenylthio)propionate

A solution of 0.203 g (1.84 mmoles) of thiophenol, 0.182 g (1.82 mmoles) of ethyl acrylate, 5 drops of Et_3N , and 5 ml of EtOH was stirred at room temperature for 3 hours. The mixture is then taken to dryness in vacuo to give 0.372 g of product (clear liquid).

30 TLC (10% EtOAc-hexane): R_f is 0.44.

$^1\text{H-NMR}$ (CDCl_3): δ 1.24, t, 3 H; 2.60, complex t 2 H; 3.16, complex t, 2 H; 4.14, q, 2H; 7.3, m, 5H.

Preparation 10 3-(Phenylthio)propionic acid

To a solution of 0.143 g (0.68 mmoles) of ethyl 3-(phenylthio)propionate in 5 ml of MeOH is added dropwise 0.816 ml (0.816 mmoles) of 1 N NaOH. After 35 minutes, MeOH is removed in vacuo and the aqueous residue is extracted with hexane and H_2O . The hexane layer is removed and discarded and the aqueous layer is acidified

-22-

with 0.27 ml of 3 N HCl. The aqueous mixture is extracted with EtOAc (backwashed with brine) and organic layers are filtered through Na_2SO_4 and taken to dryness in vacuo to give 0.1044 g (84%) of product. Crystallization from EtOAc-hexane gives (1st and 2nd crops)

5 0.066 g of the titled product, m.p. 59.5-60.0°.

TLC (20% EtOAc-hexane): slight movement off baseline.

Mass spec.: m/z at 182.

Calc'd for $\text{C}_9\text{H}_{10}\text{O}_2\text{S}$: C, 59.31; H, 5.53. Found: C, 59.02; H, 5.51.

10 $^1\text{H-NMR}$ (CDCl_3): δ 2.66, complex t, 2H; 3.16, complex t, 2H; 7.3, m, 5H.

Example 2 3-(Phenylthio)propionyl-His-Leu Ψ [CH(OH) CH_2]Val-Ile-AMP

A mixture of 0.0139 g (0.0763 mmoles) of 3-(phenylthio)propionic acid, 0.0503 g (0.0694 mmoles) of His(Tos)-Leu Ψ [CH(OH) CH_2]Val-Ile-AMP, 0.0126 ml (0.0832 mmoles) of DEPC, 0.0116 ml (0.0832 mmoles) of Et_3N , and 3 ml of CH_2Cl_2 is stirred at room temperature for 2 hours. The reaction is extracted with CHCl_3 and aq. NaHCO_3 and the organic layers are filtered through Na_2SO_4 and concentrated. CH_2Cl_2 and hexane are added to the residue and, after cooling in the refrigerator, the solvent is decanted. The remaining solid is stirred for 40 hours with 0.031 g of 1-HOBT in 30 ml of MeOH and 5 ml of CH_2Cl_2 . The solvents are then removed in vacuo and extraction with CHCl_3 -aq. NaHCO_3 is attempted. This results in a bad precipitation of product (mostly suspended in the aqueous phase). The CHCl_3 layer is removed and the aqueous suspension is centrifuged and decanted. The pellet is centrifuged twice with H_2O and the remaining solid is absorbed onto silica gel and chromatographed on silica gel (10 g) using 8% MeOH- CH_2Cl_2 (NH_4OH sat'd) to give 0.0352 g of the titled compound.

TLC (8% MeOH- CH_2Cl_2 - NH_4OH): Rf is 0.16.

30 FAB mass spec.: [M+H] measured: 736.4211; theory: 736.4220.

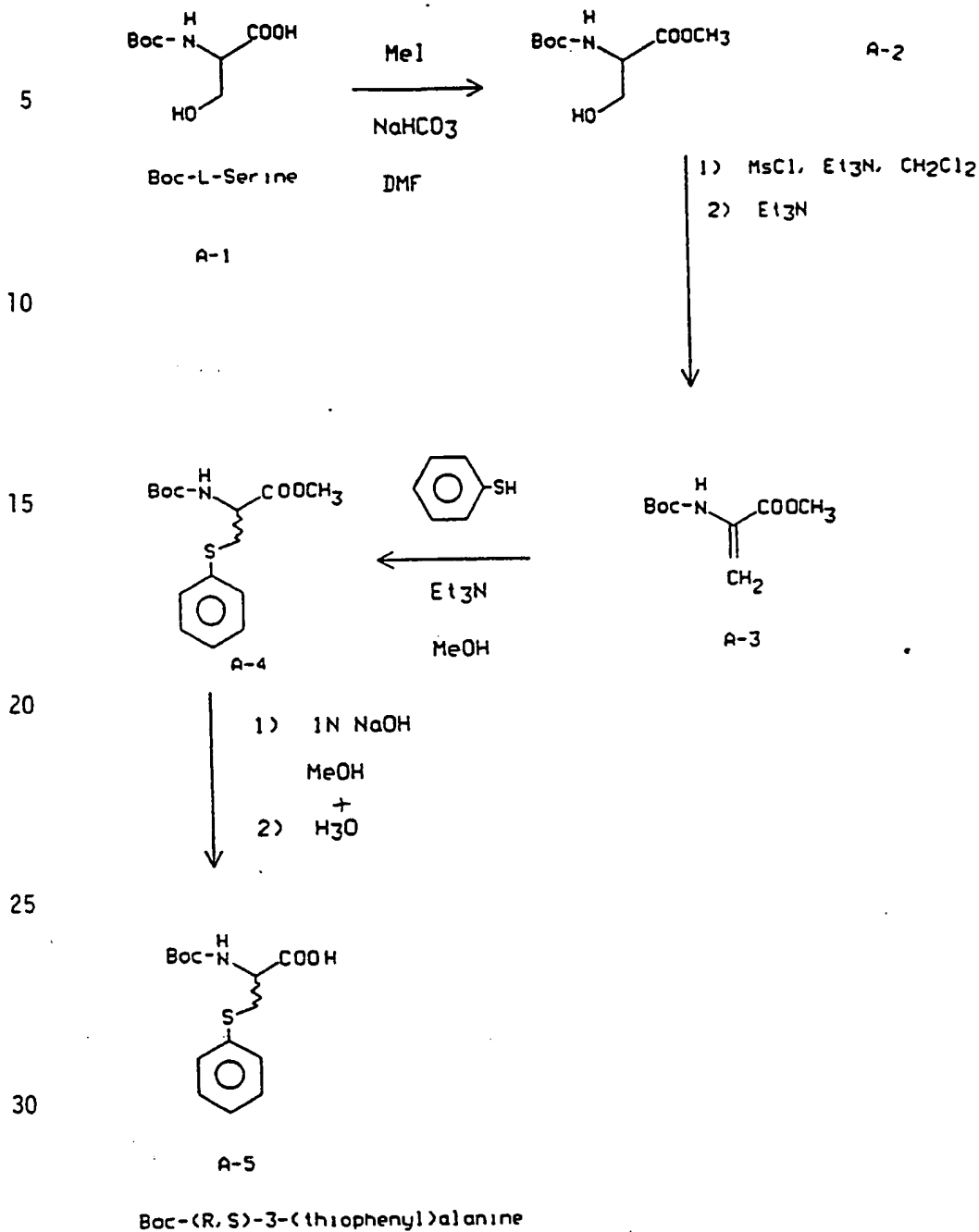
HPLC: pH 3 phosphate buffer, Brownlee C_{18} column: 60% A-40% B, k' is 5.8, 100% purity; 225 nM, A 0.1, flow rate 1.5 ml/min.

Example 3 (Phenylthio)acetyl-His-Leu Ψ [CH(OH) CH_2]Val-Ile-AMP

Using methods described herein, the titled compound is prepared.

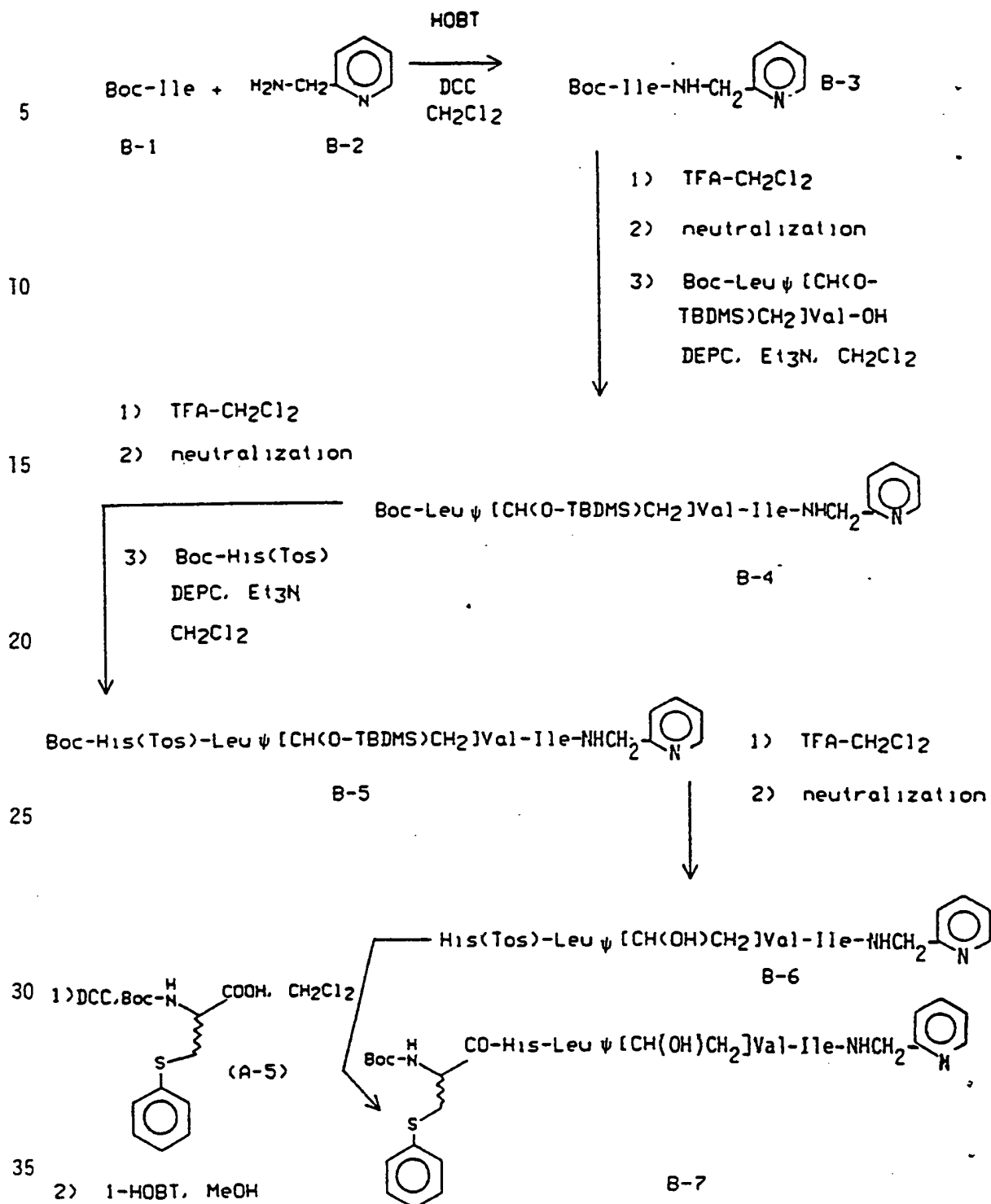
35 FAB mass spec.: [M+H] measured: 722.404;
722.4063.

-23-

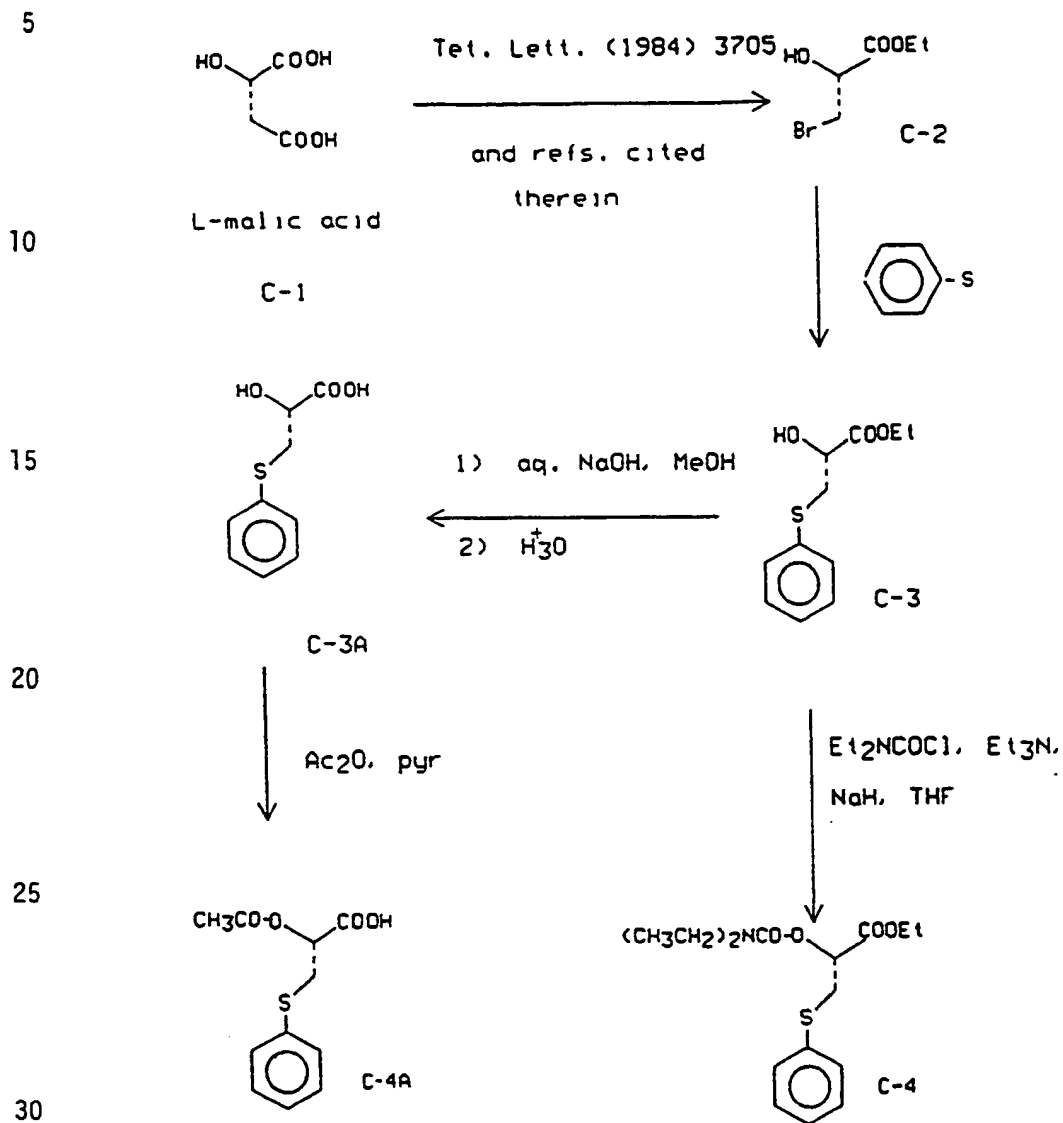
CHART A

-24-

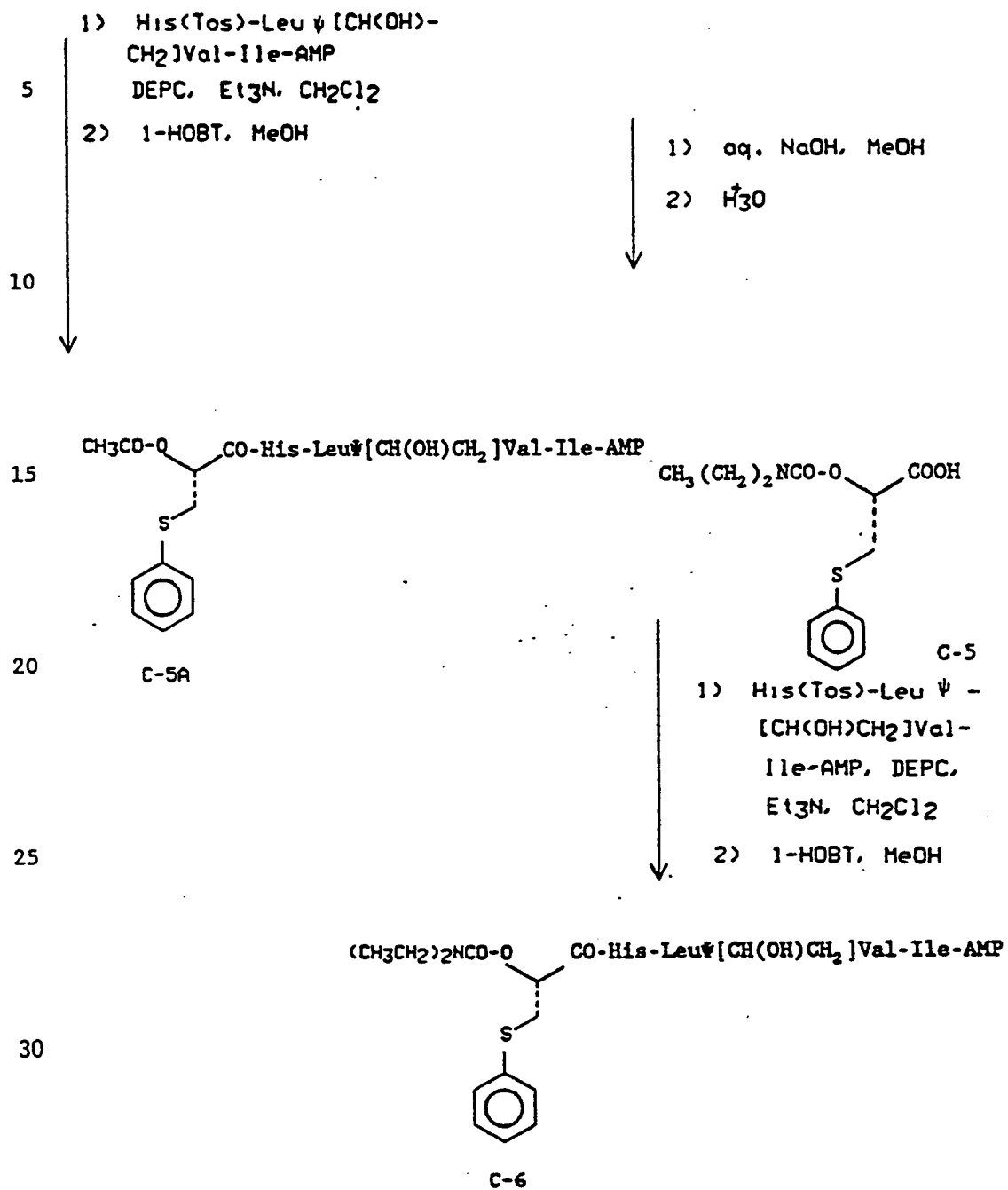
CHART B



-25-

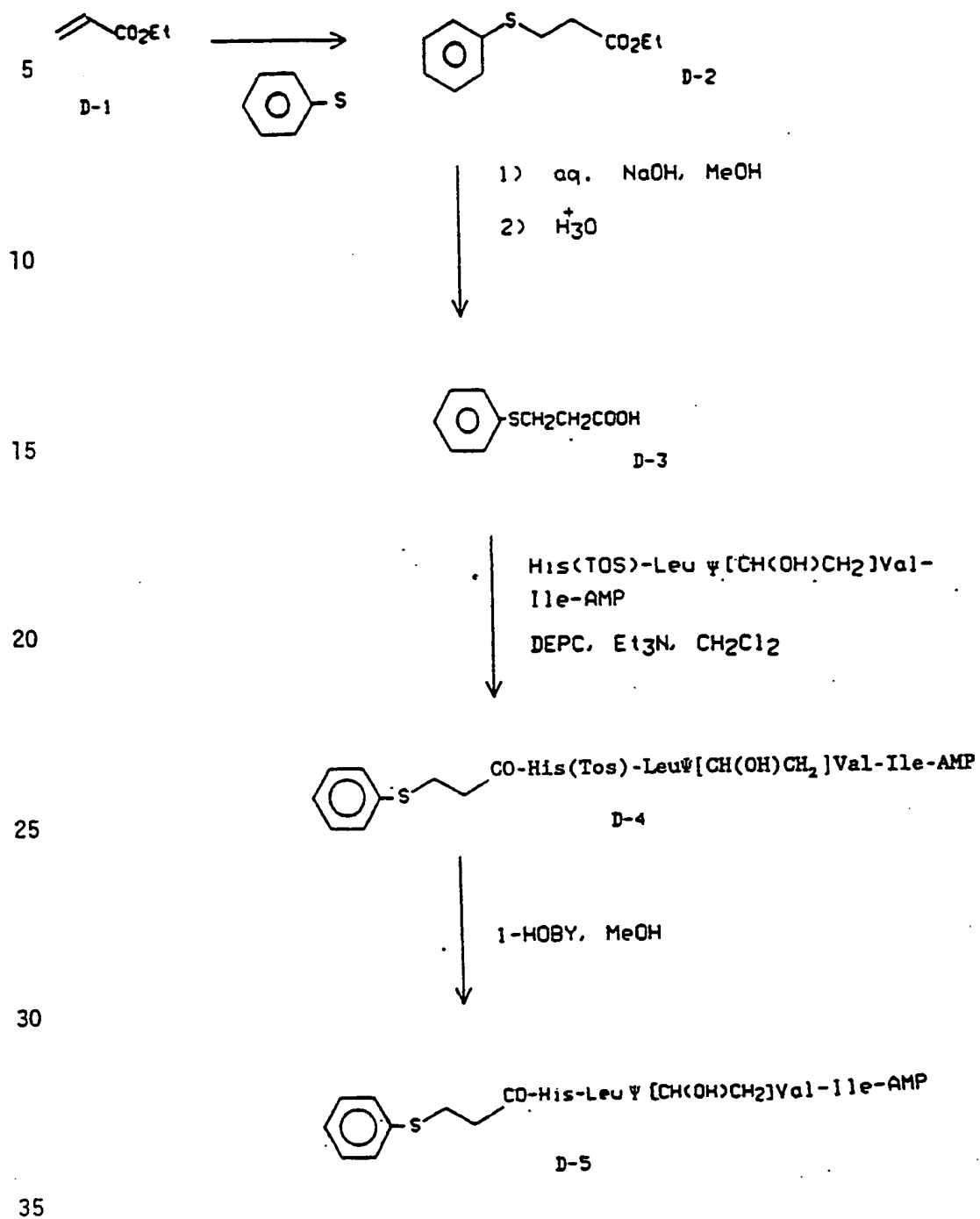
CHART C

-26-

CHART C (continued)

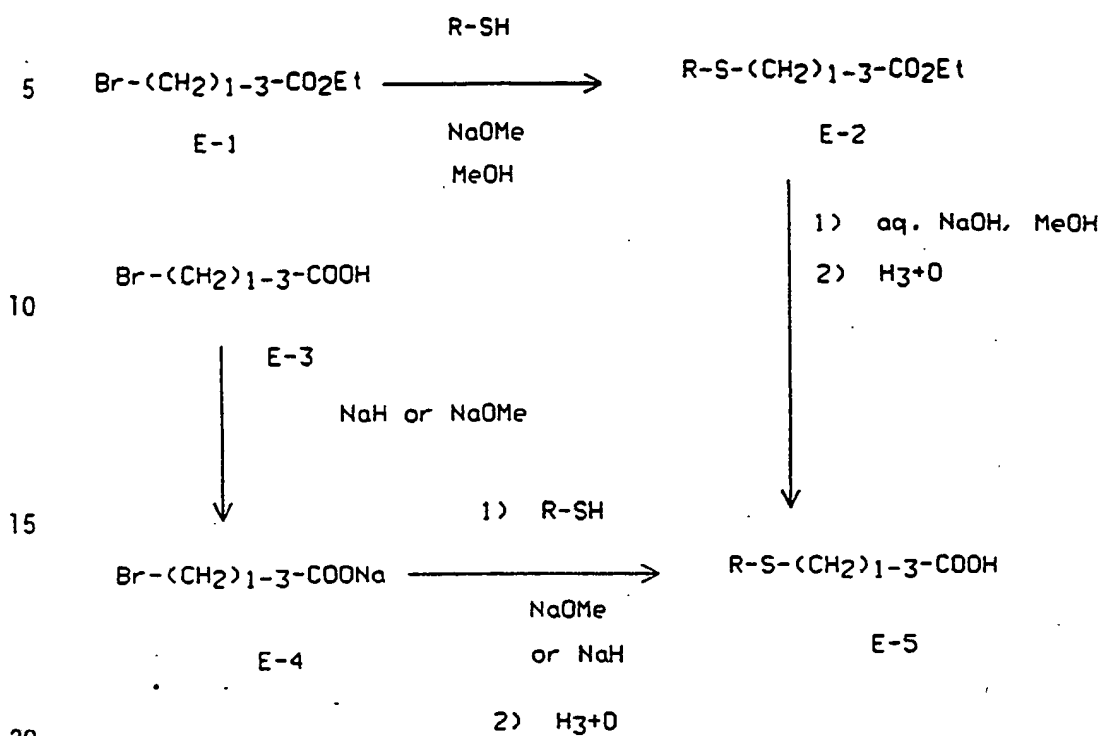
WEIGHT 175 SHEET

-27-

CHART D

SUBSTITUTE SHEET

-28-

CHART E

R = aryl, cycloalkyl, alkyl

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35

-29-

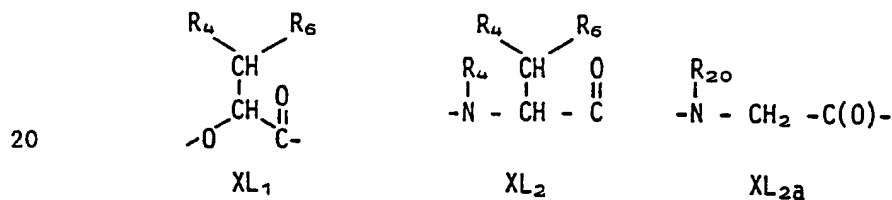
CLAIMS

1. A r nin inhibit ry peptide of the formula $X-A_6-B_7-C_8-D_9-E_1-O-F_{11}-G_{12}-H_{13}-I_{14}-Z$,

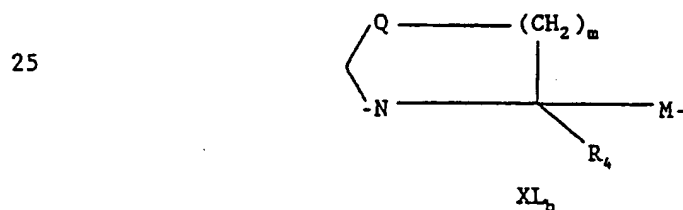
wher in X is

- 5 (a) hydrogen,
 (b) C_1-C_5 alkyl
 (c) $R_5-O-CH_2-C(=O)-$,
 (d) $R_5-CH_2-O-C(=O)-$,
 (e) $R_5-O-C(=O)-$,
 10 (f) $R_5-(CH_2)_n-C(=O)-$,
 (g) $R_4N(R_4)-(CH_2)_n-C(=O)-$,
 (h) $R_5-SO_2-(CH_2)_q-C(=O)-$,
 (i) $R_5-SO_2-(CH_2)_q-O-C(=O)-$, or
 (j) $R_5-(CH_2)_1-C(=O)-$;

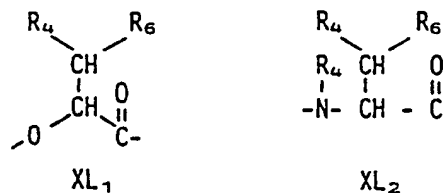
15 wherein A_6 is absent or a divalent moiety of the formula XL_1 , XL_2 , or XL_{2a}



wherein B_7 is absent or a divalent moiety of the formula XL_b

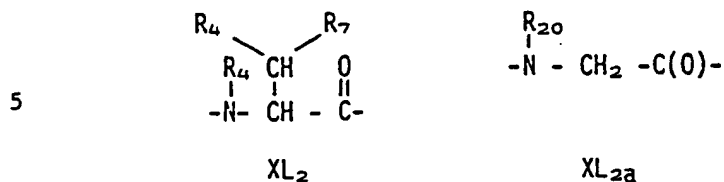


30 wherein C_8 is absent or a divalent moiety of the formula XL_1 or XL_2 ,



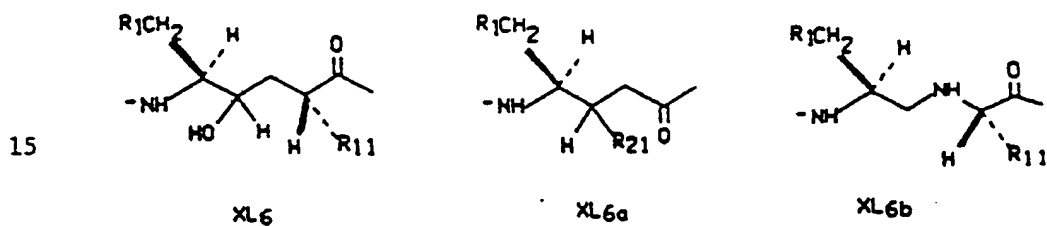
-30-

wherein D_9 is absent or a divalent moiety of the formula XL_2 or XL_{2a} ,

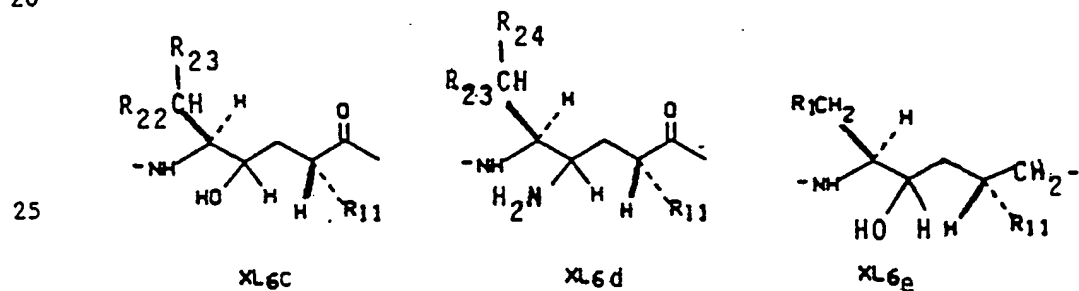


wherein E_{10} - F_{11} is a divalent moiety of the formula XL_6 , XL_{6a} , XL_{6b} , XL_{6c} , XL_{6d} , or XL_{6e} .

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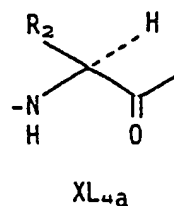
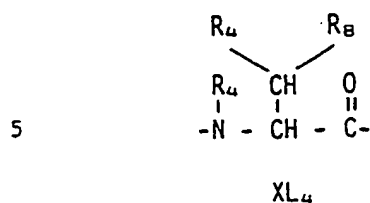


30 wherein * indicates an asymmetric center which is either in the R or S configuration;

wherein G_{12} is absent or a divalent moiety of the formula XL_4 or XL_{4a}

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-31-



10

wherein H₁₃ is absent or a divalent moiety of the formula XL₄,



wherein I₁₄ is absent or a divalent moiety of the formula XL₅,



wherein Z is

- 25
- (a) -O-R₁₀,
 - (b) -N(R₄)R₁₄, or
 - (c) C₄-C₈cyclic amino;

wherein R is

- 30
- (a) isopropyl,
 - (b) isobutyl,
 - (c) phenylmethyl, or
 - (d) C₃-C₇cycloalkyl;

wherein R₁ is

- 35
- (a) hydrogen,
 - (b) C₁-C₅alkyl,
 - (c) aryl,
 - (d) C₃-C₇cycloalkyl,
 - (e) -Het,
 - (f) C₁-C₃alkoxy, or

-32-

(g) C_1-C_3 alkylthio;wherein R_2 is

(a) hydrogen, or

(b) $-CH(R_3)R_4$;5 wherein R_3 is

(a) hydrogen,

(b) hydroxy,

(c) C_1-C_5 alkyl,(d) C_3-C_7 cycloalkyl,

10 (e) aryl,

(f) -Het,

(g) C_1-C_3 alkoxy, or(h) C_1-C_3 alkylthio;wherein R_4 at each occurrence is the same or different and is

15 (a) hydrogen, or

(b) C_1-C_5 alkyl;wherein R_5 is(a) C_1-C_6 alkyl,(b) C_3-C_7 cycloalkyl,

20 (c) aryl,

(d) -Het, or

(e) 5-oxo-2-pyrrolidinyl;

wherein R_6 is

(a) -S-aryl,

25 (b) -S- C_3-C_7 cycloalkyl, or(c) -S- C_1-C_6 alkyl;wherein R_7 is

(a) hydrogen,

(b) C_1-C_5 alkyl,

30 (c) hydroxy,

(d) amino C_1-C_4 alkyl-,(e) guanidinyl C_1-C_3 alkyl-,

(f) aryl,

(g) -Het,

35 (h) methylthio,

(i) C_3-C_7 cycloalkyl, or

(j) amin ;

wherein R_8 is

-33-

- (a) hydr gen,
 (b) C_1-C_5 alkyl,
 (c) hydr xy,
 (d) aryl,
 5 (e) -Het,
 (f) guanidiny l C_1-C_3 alkyl-, or
 (g) C_3-C_7 cycloalkyl;

wherein R_9 is

- (a) hydrogen,
 10 (b) hydroxy,
 (c) amino C_1-C_4 alkyl-, or
 (d) guanidiny l C_1-C_3 alkyl-;

wherein R_{10} is

- (a) hydrogen,
 15 (b) C_1-C_5 alkyl,
 (c) $-(CH_2)_n R_{15}$,
 (d) $-(CH_2)_n R_{17}$,
 (e) C_3-C_7 cycloalkyl,
 (f) a pharmaceutically acceptable cation,
 20 (g) $-(CHR_{25})-CH_2-R_{15}$, or
 (h) $-CH_2-(CHR_{12})-R_{15}$;

wherein R_{11} is -R or $-R_2$;wherein R_{12} is $-(CH_2)_n-R_{13}$;wherein R_{13} is

- 25 (a) aryl,
 (b) amino,
 (c) mono-, di or tri- C_1-C_3 alkylamino,
 (d) -Het,
 (e) C_1-C_5 alkyl
 30 (f) C_3-C_7 cycloalkyl,
 (g) C_1-C_5 alkenyl,
 (h) C_3-C_7 cycloalkenyl,
 (i) hydroxy,
 (j) C_1-C_3 alkoxy.
 35 (k) C_1-C_3 alkanoyloxy,
 (l) mercapto,
 (m) C_1-C_3 alkylthio,
 (n) -COOH,

-34-

- (o) $-\text{CO}-\text{O}-\text{C}_1-\text{C}_6$ alkyl,
- (p) $-\text{CO}-\text{O}-\text{CH}_2-(\text{C}_1-\text{C}_3 \text{ alkyl})-\text{N}(\text{C}_1-\text{C}_3 \text{ alkyl})_2$,
- (q) $-\text{CO}-\text{NR}_{22}\text{R}_{26}$;
- (r) C_4-C_7 cyclic amino,
- 5 (s) C_4-C_7 cycloalkylamino,
- (t) guanidyl,
- (u) cyano,
- (v) N-cyanoguanidyl,
- (w) cyanoamino,
- 10 (x) (hydroxy C_2-C_4 alkyl)amino, or
- (y) di-(hydroxy C_2-C_4 alkyl)amino;

wherein R_{14} is

- (a) hydrogen,
- (b) C_1-C_{10} alkyl,
- 15 (c) $-(\text{CH}_2)_n-\text{R}_{18}$,
- (d) $-(\text{CH}_2)_n-\text{R}_{19}$,
- (e) $-(\text{CHR}_{25})-\text{CH}_2-\text{R}_{15}$,
- (f) $-\text{CH}_2-(\text{CHR}_{12})-\text{R}_{15}$,
- (g) (hydroxy C_1-C_8 alkyl), or
- 20 (h) $(\text{C}_1-\text{C}_3 \text{ alkoxy})\text{C}_1-\text{C}_8$ alkyl;

wherein R_{15} is

- (a) hydroxy,
- (b) C_3-C_7 cycloalkyl,
- (c) aryl,
- 25 (d) amino,
- (e) mono-, di-, or tri- C_1-C_3 alkylamino,
- (f) mono- or di-[hydroxy C_2-C_4 alkyl]amino,
- (g) -Het,
- (h) C_1-C_3 alkoxy-,
- 30 (i) C_1-C_3 alkanoyloxy-,
- (j) mercapto,
- (k) C_1-C_3 alkylthio-,
- (l) C_1-C_3 alkyl,
- (m) C_4-C_7 cyclic amino,
- 35 (n) C_4-C_7 cycloalkylamino,
- (o) C_1-C_3 alkenyloxy,
- (p) C_3-C_7 cycloalkenyl;

wherein R_{16} is

-35-

- (a) aryl,
- (b) amin ,
- (c) mono- r di- C₁-C₃alkylamino,
- (d) hydroxy,
- 5 (e) C₃-C₇cycloalkyl,
- (f) C₄-C₇cyclic amino, or
- (g) C₁-C₃alkanoyloxy;

wherein R₁₇ is

- (a) -Het,
- 10 (b) C₁-C₅alkenyl,
- (c) C₃-C₇cycloalkenyl,
- (d) C₁-C₃alkoxy,
- (e) mercapto,
- (f) C₁-C₃alkylthio,
- 15 (g) -COOH,
- (h) -CO-O-C₁-C₆alkyl,
- (i) -CO-O-CH₂-(C₁-C₃alkyl)-N(C₁-C₃alkyl)₂,
- (j) -CO-NR₂₂R₂₆,
- (k) tri-C₁-C₃alkylamino,
- 20 (l) guanidyl,
- (m) cyano,
- (n) N-cyanoguanidyl,
- (o) (hydroxy C₂-C₄alkyl)amino,
- (p) di-(hydroxy C₂-C₄alkyl)amino, or
- 25 (q) cyanoamino;

wherein R₁₈ is

- (a) amino,
- (b) mono-, or di- C₁-C₃alkylamino,
- (c) C₄-C₇cyclic amino, or
- 30 (d) C₄-C₇cycloalkylamino;

wherein R₁₉ is

- (a) aryl,
- (b) -Het,
- (c) tri-C₁-C₃alkylamino,
- 35 (d) C₃-C₇cycloalkyl,
- (e) C₁-C₅alkenyl,
- (f) C₃-C₇cycloalkenyl,
- (g) hydroxy,

-36-

- (h) C_1-C_3 alk xy,
 (i) C_1-C_3 alkan yloxy,
 (j) mercapto,
 (k) C_1-C_3 alkylthio,
 5 (l) $-COOH$,
 (m) $-CO-O-C_1-C_8$ alkyl,
 (n) $-CO-O-CH_2-(C_1-C_3 \text{ alkyl})-N(C_1-C_3 \text{ alkyl})_2$,
 (o) $-CO-NR_{22}R_{26}$,
 (p) guanidyl,
 10 (q) cyano,
 (r) N-cyanoguanidyl,
 (s) cyanoamino,
 (t) (hydroxy C_2-C_4 alkyl)amino,
 (u) di-(hydroxy C_2-C_4 alkyl)amino; or
 15 (v) $-SO_3H$;

wherein R_{20} is

- (a) hydrogen,
 (b) C_1-C_3 alkyl, or
 (c) aryl- C_1-C_3 alkyl;

20 wherein R_{21} is

- (a) $-NH_2$, or
 (b) $-OH$;

wherein R_{22} is

- (a) hydrogen, or
 25 (b) C_1-C_3 alkyl;

wherein R_{23} is

- (a) $-(CH_2)_n-OH$,
 (b) $-(CH_2)_n-NH_2$,
 (c) aryl, or
 30 (d) C_1-C_3 alkyl;

wherein R_{24} is

- (a) $-R_1$,
 (b) $-(CH_2)_n-OH$, or
 (c) $-(CH_2)_n-NH_2$;

35 wherein R_{25} is $-(CH_2)_n-R_{13}$;

wherein R_{26} is

- (a) hydr gen,
 (b) C_1-C_3 alkyl, r

-37-

(c) phenyl- C_1-C_3 alkyl;

wherein i is one to three, inclusive;

wherein m is one or two;

wherein for each occurrence n is independently an integer of

5 zero to five, inclusive;

wherein p is zero to 2 inclusive;

wherein q is 1 to 5, inclusive;

wherein Q is

- (a) $-CH_2-$,
- 10 (b) $-CH(OH)-$,
- (c) $-O-$, or
- (d) $-S-$; and

wherein M is

- (a) $-CO-$, or
- 15 (b) $-CH_2-$;

wherein aryl is phenyl or naphthyl substituted by zero to 3 of
the following:

- (a) C_1-C_3 alkyl,
- (b) hydroxy,
- 20 (c) C_1-C_3 alkoxy,
- (d) halo,
- (e) amino,
- (f) mono- or di- C_1-C_3 alkylamino,
- (g) $-CHO$,
- 25 (h) $-COOH$,
- (i) $COOR_{26}$,
- (j) $CONHR_{26}$,
- (k) nitro,
- (l) mercapto,
- 30 (m) C_1-C_3 alkylthio,
- (n) C_1-C_3 alkylsulfinyl,
- (o) C_1-C_3 alkylsulfonyl,
- (p) C_1-C_3 alkylsulfonyl- $N(R_4)-$
- (q) SO_3H ,
- 35 (r) SO_2NH_2 ,
- (s) $-CN$, or
- (t) $-CH_2NH_2$;

-38-

wherein -Het is a 5- or 6-membered saturated or unsaturated ring containing from one to three heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur; and including any bicyclic group in which any of the above heterocyclic rings is fused to a benzene ring, which heterocyclic moiety is substituted with zero to 3 of the following:

- (i) C₁-C₈ alkyl,
- (ii) hydroxy,
- (iii) trifluoromethyl,
- 10 (iv) C₁-C₄ alkoxy,
- (v) halo,
- (vi) aryl,
- (vii) aryl C₁-C₄ alkyl-,
- (viii) amino, and
- 15 (ix) mono- or di-C₁-C₄ alkylamino;

with the overall provisos that

- (1) X is R₆-(CH₂)₁-C(=O)- only when A₆, B₇, and C₈ are absent;
- (2) R₁₈ or R₁₉ is hydroxy, mercapto, or amino, or a mono-substituted nitrogen containing group bonded through the nitrogen only when n is not one;
- (3) R₁₂ is -(CH₂)_n-R₁₃ and n is zero and both R₁₃ and R₁₄ are oxygen-, nitrogen-, or sulfur-containing substituents bonded through the hetero atom, only when the hetero atom is not also bonded to hydrogen;
- 25 (4) R₂₅ is -(CH₂)_n-R₁₃ and n is zero only when R₁₃ is other than a primary or secondary nitrogen-containing group, hydroxy or mercapto group or when R₄ of -N(R₄)R₁₄ is other than hydrogen; and
- (5) R₁₇ or R₁₈ is -COOH only when n for that moiety is other than zero; and
- 30 or a carboxy-, amino-, or other reactive group-protected form or a pharmaceutically acceptable acid addition salt thereof.

2. A compound of claim 1, wherein A, B, H, and I are absent, and E₁₀F₁₁ is XL₈.

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3. Boc-((S-phenyl)-DL-Cys)-His-LeuY[CH(OH)CH₂]Val-Ile-AMP or N-[4-[[N-[N-(1-L-arginyl-L-prolyl)-L-phenylalanyl]-L-histidyl]amino]-3-

-39-

hydr xy-6-methyl-1-oxoheptyl]-L-isol ucine, tris(triflu r acetate)
(salt), a compound of claim 2.

4. 3-(Phenylthio)propionyl-His-Leu Ψ [CH(OH)CH₂]Val-Ile-AMP, or N-(2-
5 hydroxy-5-methyl-1-(2-methylpropyl)-4-(((2-methyl-1-(((2-pyridinyl-
methyl)amino)carbonyl)butyl)amino)carbonyl)hexyl)- α -(1-oxo-3-
(phenylthio)propyl)amino)-1H-imidazole-4-propanamide, a compound of
claim 2.
- 10 5. (Phenylthio)acetyl-His-Leu Ψ [CH(OH)CH₂]Val-Ile-AMP, a compound of
claim 2.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 86/02227

| I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC ⁴ : C 07 K 5/06; C 07 D 233/54; C 07 K 5/10; //A 61 K 37/64; A 61 K 37/02 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--|--|-------------------------------------|--|--|---|---|--|-----|---|--|-----|-----|--|-----|---|--|-----|---|---|-----|---|---|-----|---|---|-----|---|--|
| II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched ⁷</div> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%; text-align: left; border-bottom: 1px solid black;">Classification System ¹</th> <th style="width: 75%; text-align: left; border-bottom: 1px solid black;">Classification Symbols</th> </tr> <tr> <td style="padding: 5px;">IPC ⁴</td> <td style="padding: 5px;">C 07 K 5/00; C 07 D 233/00; A 61 K 37/00;</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched ⁸</div> | | | Classification System ¹ | Classification Symbols | IPC ⁴ | C 07 K 5/00; C 07 D 233/00; A 61 K 37/00; | | | | | | | | | | | | | | | | | | | | | | |
| Classification System ¹ | Classification Symbols | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| IPC ⁴ | C 07 K 5/00; C 07 D 233/00; A 61 K 37/00; | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; text-align: left; border-bottom: 1px solid black;">Category ⁹</th> <th style="width: 70%; text-align: left; border-bottom: 1px solid black;">Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²</th> <th style="width: 20%; text-align: left; border-bottom: 1px solid black;">Relevant to Claim No. ¹³</th> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">EP, A, 0114993 (MERCK) 8 August 1984, see page 40, example 26; pages 47-51; claim 1;</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1,2</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">see page 6, formula 1, lines 10-18 cited in the application --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">3-5</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X,Y</td> <td style="padding: 5px;">WO, A, 84/3044 (FERRING A.B.) 16 August 1984, see page 21, example 6; claims 1-2; pages 50-56 & EP, A, 0118223 (cited in the application) --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-5</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">X</td> <td style="padding: 5px;">EP, A, 0156322 (MERCK) 2 October 1985, see page 69, example 17; pages 70-78; claim 1 --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1,2</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">EP, A, 0157409 (MERCK) 9 October 1985, see page 55, lines 1-20 --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-5</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">EP, A, 0143746 (CIBA-GEIGY) 5 June 1985, see page 144, claim 1 --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-5</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;">GB, A, 2045771 (SQUIBB) 5 November 1980, see page 1, lines 1-35 . /.</td> <td style="text-align: center; vertical-align: top; padding: 5px;">3-5</td> </tr> </table> <div style="font-size: small; padding: 5px;"> <table style="width: 100%;"> <tr> <td style="vertical-align: top; width: 50%;"> ¹⁰ Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed </td> <td style="vertical-align: top; width: 50%;"> "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family </td> </tr> </table> </div> | | | Category ⁹ | Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³ | X | EP, A, 0114993 (MERCK) 8 August 1984, see page 40, example 26; pages 47-51; claim 1; | 1,2 | Y | see page 6, formula 1, lines 10-18 cited in the application -- | 3-5 | X,Y | WO, A, 84/3044 (FERRING A.B.) 16 August 1984, see page 21, example 6; claims 1-2; pages 50-56 & EP, A, 0118223 (cited in the application) -- | 1-5 | X | EP, A, 0156322 (MERCK) 2 October 1985, see page 69, example 17; pages 70-78; claim 1 -- | 1,2 | Y | EP, A, 0157409 (MERCK) 9 October 1985, see page 55, lines 1-20 -- | 1-5 | Y | EP, A, 0143746 (CIBA-GEIGY) 5 June 1985, see page 144, claim 1 -- | 1-5 | A | GB, A, 2045771 (SQUIBB) 5 November 1980, see page 1, lines 1-35 . /. | 3-5 | ¹⁰ Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family |
| Category ⁹ | Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³ | | | | | | | | | | | | | | | | | | | | | | | | | | |
| X | EP, A, 0114993 (MERCK) 8 August 1984, see page 40, example 26; pages 47-51; claim 1; | 1,2 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Y | see page 6, formula 1, lines 10-18 cited in the application -- | 3-5 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| X,Y | WO, A, 84/3044 (FERRING A.B.) 16 August 1984, see page 21, example 6; claims 1-2; pages 50-56 & EP, A, 0118223 (cited in the application) -- | 1-5 | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| Y | EP, A, 0157409 (MERCK) 9 October 1985, see page 55, lines 1-20 -- | 1-5 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Y | EP, A, 0143746 (CIBA-GEIGY) 5 June 1985, see page 144, claim 1 -- | 1-5 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| A | GB, A, 2045771 (SQUIBB) 5 November 1980, see page 1, lines 1-35 . /. | 3-5 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ¹⁰ Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| IV. CERTIFICATION <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; padding: 5px;"> Date of the Actual Completion of the International Search <div style="text-align: center; font-weight: bold;">29th January 1987</div> </td> <td style="width: 50%; padding: 5px;"> Date of Mailing of this International Search Report <div style="text-align: center; font-weight: bold;">27 MAR 1987</div> </td> </tr> <tr> <td style="width: 50%; padding: 5px;"> International Searching Authority <div style="text-align: center; font-weight: bold;">EUROPEAN PATENT OFFICE</div> </td> <td style="width: 50%; padding: 5px;"> Signature of Authorized Officer <div style="text-align: center;">M. VAN MOL </div> </td> </tr> </table> | | | Date of the Actual Completion of the International Search <div style="text-align: center; font-weight: bold;">29th January 1987</div> | Date of Mailing of this International Search Report <div style="text-align: center; font-weight: bold;">27 MAR 1987</div> | International Searching Authority <div style="text-align: center; font-weight: bold;">EUROPEAN PATENT OFFICE</div> | Signature of Authorized Officer <div style="text-align: center;">M. VAN MOL </div> | | | | | | | | | | | | | | | | | | | | | | |
| Date of the Actual Completion of the International Search <div style="text-align: center; font-weight: bold;">29th January 1987</div> | Date of Mailing of this International Search Report <div style="text-align: center; font-weight: bold;">27 MAR 1987</div> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| International Searching Authority <div style="text-align: center; font-weight: bold;">EUROPEAN PATENT OFFICE</div> | Signature of Authorized Officer <div style="text-align: center;">M. VAN MOL </div> | | | | | | | | | | | | | | | | | | | | | | | | | | | |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) | | |
|--|--|----------------------|
| Category * | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No |
| A | Chemical Abstracts, volume 98, no. 25, 20 June 1983, (Columbus, Ohio, US), Kurihara, Tozaburo et al.: "Studies on antibacterial peptide. XVII. Synthesis and antibacterial activity of cyclic alpha-acylpentapeptides related to colistin", see page 597, abstract 215980d, & Yakugaku Zasshi 1983, 103(1), 80-4 | 3-5 |
| P, X | EP, A, 0173481 (UPJOHN) 5 March 1986, see page 162, claim 11 | 1, 2 |
| Y | see pages 148-159, claim 1 | 3-5 |
| ----- | | |

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO.

PCT/US 86/02227 (SA 15091)

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 27/02/87

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| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|--|---------------------|----------------------------|---------------------|
| EP-A- 0114993 | 08/08/84 | JP-A- 59155345 | 04/09/84 |
| WO-A- 843044 | | None | |
| EP-A- 0156322 | 02/10/85 | JP-A- 60222452 | 07/11/85 |
| EP-A- 0157409 | 09/10/85 | JP-A- 60231695 | 18/11/85 |
| EP-A- 0143746 | 05/06/85 | AU-A- 3580484 | 30/05/85 |
| | | JP-A- 60136594 | 20/07/85 |
| | | US-A- 4613676 | 23/09/86 |
| GB-A- 2045771 | 05/11/80 | BE-A- 882601 | 02/10/80 |
| | | FR-A, B 2453135 | 31/10/80 |
| | | NL-A- 8001675 | 06/10/80 |
| | | DE-A- 3012140 | 23/10/80 |
| | | LU-A- 82316 | 16/12/80 |
| | | AU-A- 5638080 | 09/10/80 |
| | | JP-A- 55133345 | 17/10/80 |
| | | SE-A- 8002512 | 03/10/80 |
| | | AU-B- 537592 | 05/07/84 |
| | | CH-A- 645092 | 14/09/84 |
| EP-A- 0173481 | 05/03/86 | JP-A- 61063641 | 01/04/86 |

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see Official Journal of the European Patent Office, No. 12/82